A MORPHOLOGICAL STUDY OF THE ALGAL SYMBIONTS OF FOUR TAIWAN LICHENS: *ANAPTYCHIA COMOSA, A. DENDRICATA, PARMELIA CAPERATA,* AND *P. RUDECTA.*[1]

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ABSTRACT

Three species of the phycobiont *Trebouxia* have been described in this investigation. On the basis of this study, all the algal symbionts isolated from the four species of lichens, namely, *Anaptychia comosa*, *A. dendricata*, *Parmelia caperata*, and *P. rudecta*, collected in Taiwan, belong to *Trebouxia* Group II. The results show that different species of lichens contain the morphologically identical algae (e. g. symbionts of *A. comosa*, and *A. dendricata*,) and that at times the same species of lichen may contain physiologically different forms of algal symbiont (e. g. *P. caperata* as compared in this study with my previous study, Wang-Yang, 1965). However, at the species level, lichen fungi are not highly specific with regard to their algal partners.

INTRODUCTION

The experimental observations described in this paper deal with the cultural morphology of the algal symbionts (=phycobionts) of four lichens, namely, *Anaptychia comosa* (Eschw.) Mass., *A. dendricata* (Pers.) Vain., *Parmelia caperata* (L.) Ach. and *P. rudecta* (L.) Ach., which were used in this investigation to identify the isolated cultural phycobionts were the shape and size of the vegetative cells, the type of chromatophore, the manner of cell division, the number of aplanospores, type of zoospores and the presence or absence of a gelatinous sheath.

Previous studies on the cultural morphology of the algal symbionts of lichens (Wang-Yang, 1965) indicated that, at the species level, lichen fungi were not specific toward their algal symbionts. It is hoped that this report will contribute to the understanding of the specificity of lichens with regard to their algal symbionts.

MATERIALS AND METHODS

A. Organisms:

Fresh specimens of lichens of *Anaptychia dendricata*[3], *Parmelia caperata* (L.) Ach,[4] *P. rudecta* (L.) Ach.[5] collected from Mt. Ali and *A. comosa*[6] collected from Yang-Ming Shan were used.

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(3) (4) (6) Based on herbarium specimens determined by the author.

(5) Specimens determined by I. Sasaki.

B. Isolation and Culture:

The isolation technique of lichen algae and the method and media have been described in earlier studies (Ahmadjian 1959b, Wang-Yang 1965). Briefly, the algae were isolated from the lichen thallus by means of a micropipette. A single cell with its attached fungal hyphae was picked up with a micropipette and the cell was transferred through successive drops of sterile water until it was clear of surrounding debris, and then inoculated onto agar medium. In this investigation, the Trebouxia agar medium (Starr, 1960) generally used for such isolations was used. Its composition is as follows: each 1000 ml of medium required 850 ml Bristol's solution, 140 ml soil water, 10 g. Bacto peptone, 20 g. dextrose and 15 g. agar. Bristol's solution (modified from Bold, 1949) was used as the mineral medium. Major elements were supplied by their salts in the following amounts per liter of final solution: $NaNO_3$ 250 mg, KH_2PO_4 175 mg, K_2HPO_4 75 mg, $CaCl_2$ 25 mg, $MgSO_4 \cdot 7H_2O$ 75 mg, NaCl 25 mg. The composition of soil water is as follows (Starr, 1960): Distilled water 170 ml, stock soil 30 ml and KNO_3 (5%) 2 ml. Stock soil was obtained by mixing 500 g of sifted soil and 500 ml of distilled water. This suspension was autoclaved for 1 hour, allowed to cool and was then filtered.

For each lichen species an average of 20 algal isolations were made. These were grown in cotton-stoppered (15×150 mm) test tubes which were kept in incubation condition of 20±1°C, under an alternate 8 hours light, 16 hours dark cycle at an illumination of 50–100 foot candles. Light was supplied by cool white fluorescent bulbs. The percentage of colonies obtained ranged from 0 to 80%. Depending on the species of *Trebouxia*, the colonies became visible between 3 to 8 weeks. With the use of this isolation technique one could obtain pure clones of lichen algae.

After development of the colonies, a portion of the algal colony was inoculated into liquid media which was used to study the morphological differences of the cells. Liquid cultures were grown in cotton stoppered, 125 ml Erlenmeyer flasks which contained 30 ml of medium. One set of cultural colonies was kept in complete darkness and the other in the light. These were used for a comparative study of the morphological features of light and dark grown cultural colonies. The

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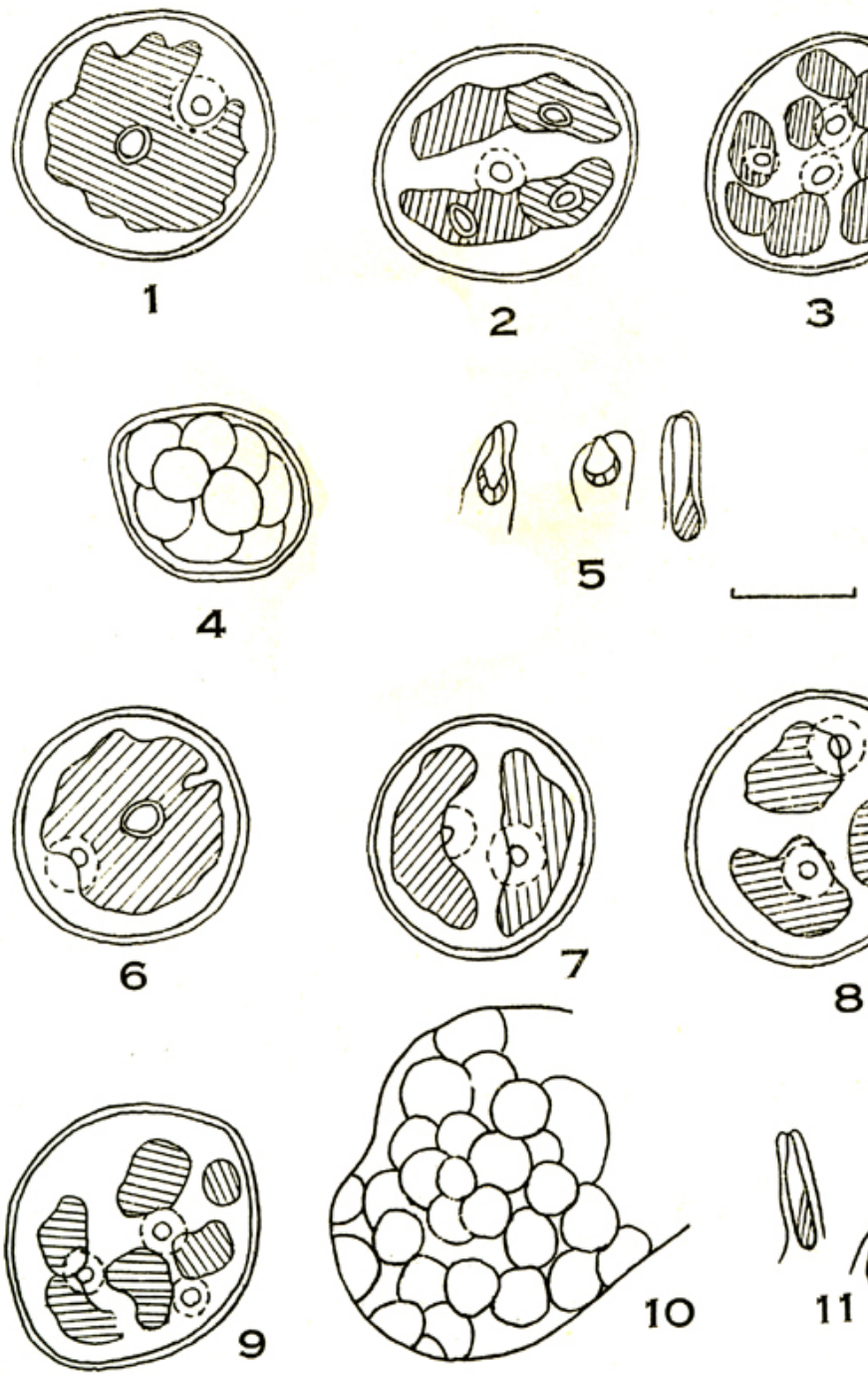
Explanation of Figures

Plate 1. Camera lucida drawings of *Trebouxia* Group II.

Fig. 1. Vegetative cell with large, central chromatophore containing 1 pyrenoid, nucleus.
Figs. 2-3. Stages in the division of the chromatophore.
Fig. 4. Aplanosporangium.
Fig. 5. Flattened and rounded zoospores.
Fig. 6. Vegetative cell.
Fig. 7. Cell showing first cleavage of chromatophore.
Figs. 8-9. Stages in the division of the chromatophore.
Fig. 10. Ruptured aplanosporangium.
Fig. 11. Zoospores.
Unit=10 microns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stages in the life history of the species were studied with the aid of hanging drop preparations. A camera lucida was used for the drawing of the different stages of the life history of cells. The presence of cellular gelatinous sheath was determined by means of India ink preparations.

RESULTS

Description of the isolated algal symbiont: The phycobionts isolated from specimens of the lichens of *A. comosa*, *A. dendricata*, *P. caperata*, *P. rudecta*, belong to *Trebouxia*[1][2] genus.

1. Phycobionts of *Anaptychia comosa*. (Cult. Coll. No.[3] 191, 195):
Trebouxia impressa Group II. (Pl. 1, Figs 1–5) (Ahmadjian 1960: 681).

Vegetative cells are spherical, and the sizes of mature vegetative cells range from 19.0×19.0 microns to 23.0×23.0 microns. The chromatophore is central and has a smooth margin. A gelatinous sheath was seen only around cells grown in organic nutrient shake culture. Aplanospores numbered mostly 8 to 32 in a sporangium. Vegetative cells are generally free-living. Motile zoospores[4] are produced and abundantly. Flattened zoospores measured 8.0 microns long by 3.0 microns wide on the average. The color of the algal colony is dark green, both in the light and dark grown cultures. The 3 month old colonies (Pl. 3, Fig. 2) have a smooth, non-granular appearance and vermiform surface. Approximately, the same growth[5] rate occurs both in the light and dark under the same cultural conditions.

(1) *Trebouxia* is characterized by the possession of a single, large, central chromatophore having one (rarely more) centrally located pyrenoid which is sometimes diffuse or consists of short radially arranged rods; production of biflagellate protosiphone type zoospores (Starr, 1955) which are naked and round up quickly in an amoeboid fashion when they come to rest. The zoospores lack a stigma, are usually markedly flattened, have a small, posterior, cup-shaped, pale-green chromatophore, and possess 2 flagella of equal length which generally extend several microns beyond the body. There is a production of aplanospores. Ahmadjian (1960) divided the species of *Trebouxia* phycobionts into 2 groups based on the nature of the chromatophores and the shape of the vegetative cells. In the first group, the chromatophore assumes a parietal, compressed configuration during division, chromatophore deeply incised, vegetative cells generally ellipsoidal. In the second group, the chromatophore is comparatively smooth-margined, vegetative cells generally spherical.

(2) All measurements and descriptions of cell shape were made from liquid cultures.

(3) The number refers to the culture.

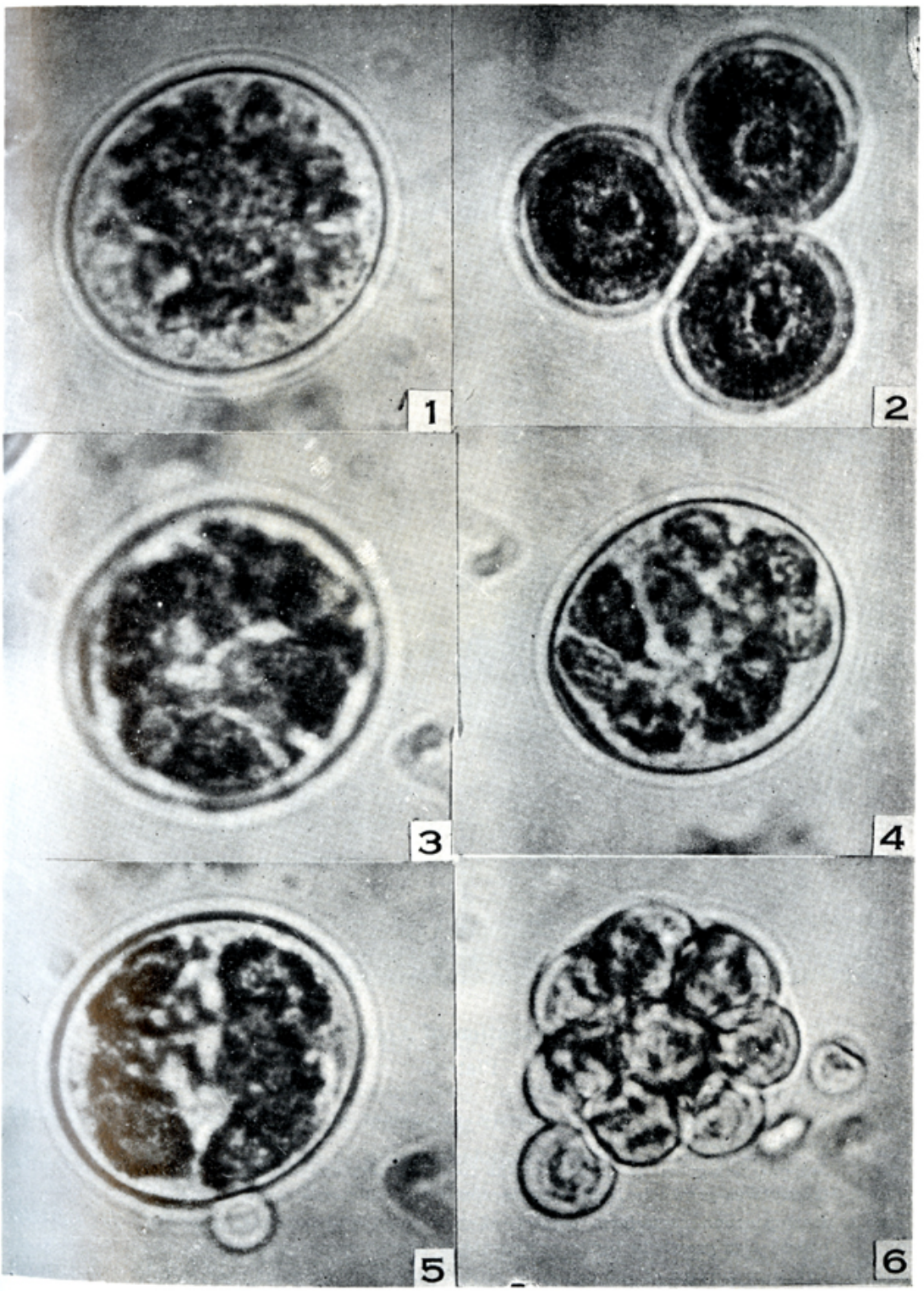
(4) The motility of the zoospores was inhibited by the addition of cotton fibers to the drop of algal suspension which was being examined microscopically. This slowed the movement of the zoospores sufficiently to allow them to be measured.

(5) Growth was determined by direct measurements of the height and width of colonies.

Explanation of Figures

Plate 2.

Fig. 1. Vegetative cell of *Trebouxia* Group II. ×1150.
Fig. 2. Vegetative cells in grouping ×1100.
Figs. 3-5. Stages in the division of the chromatophore ×1100.
Fig. 6. Ruptured aplanospores ×1500.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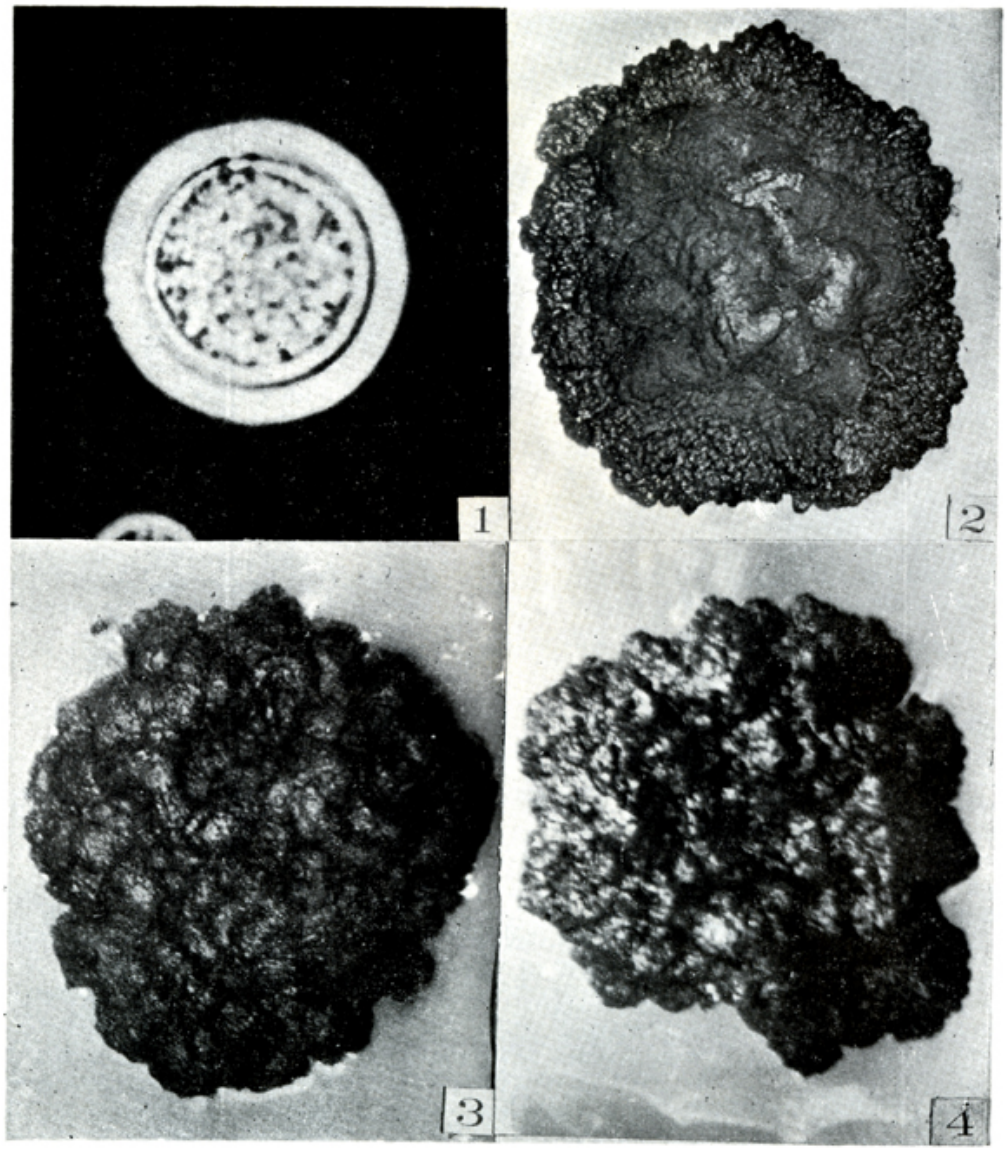

Explanation of Figures

Plate 3.

Fig. 1. Vegetative cell of *Parmelia caperata*, phycobiont in India ink preparation illustrating gelatinous sheath ×1100.

Fig. 2. Smooth, non-granular surface of the colony of phycobionts from *Anaptychia dendricata*, colony was grown in light; photographed after 3 month' growth. ×1.5 natural size.

Fig. 3. Homogeneous vermiform surface of the colony of phycobionts from *Parmelia caperata*, colony was grown in light; photographed after 3 month' growth. ×1.5 natural size.

Fig. 4. Granular surface of the colony of phycobionts from *Parmelia rudecta*, colony was grown in light; photographed after 3 month' growth. ×1.5 natural size.

2. Phycobionts of *Anaptychia dendricata*. (Cult. Coll. No. 145):
Trebouxia impressa Group II. (Ahmadjian 1960: 681)

Vegetative cells are spherical, and the size of mature vegetative cells ranged from 16.0×16.0 microns to 21.0×21.0 microns. The chromatophore is central and has a smooth margin. A gelatinous sheath was seen under all cultural conditions. Aplanospores numbered 8–32, but could go as high as large balls of aplanospores in a sporangium. Vegetative cells are free-living, but few in groups. Flattened zoospores measured 8.0 microns long by 4 microns wide on the average. The color of the algal colony is dark green both in the light and dark grown cultures. The growth rate in the light, under the same cultural condition, is about one half greater than in the dark. The stages of the life history of *Trebouxia* phycobionts from this lichen are shown on pl. 2, figs. 1–6.

3. Phycobionts of *Parmelia caperata* (L.) Ach. (Cult. Coll. No. 187):
Trebouxia gelatinosa Group II. (Pl. 1, Figs. 6–11) (Ahmadjian 1959b)

The vegetative cells are spherical and have an average diameter of 12.0×12.0 microns to 24.0×24.0 microns. The chromatophore has a smooth margin and with a central position during cell division. A distinctive gelatinous sheath (pl. 3, fig. 1) and thickening of the cell wall are present under all of the cultural conditions. The sheath varies in width from 1 to 3 microns. Large balls of aplanospores are produced in culture. Flattened zoospores are produced, and measured 8.7 microns long by 3.8 microns wide. The colony is dark green, extremely moist and has a homogeneous vermiform structure (pl. 3, fig. 3) both in the light and dark grown cultures. The growth rate in the light, under the same cultural conditions, is about one third greater than in the dark.

4. Phycobionts of *Parmelia rudecta* (L.) Ach. (Cult. Coll. No. 143):
Trebouxia anticipata Group II. (Ahmadjian 1959b)

The vegetative cell is spherical, with a smooth margined chromatophore. Sizes of mature vegetative cells range from 23.0×23.0 microns to 30.0×30.0 microns. No gelatinous sheath could be demonstrated. Numerous, large balls of aplanospores are produced in culture. The colony is dark green, having a granular surface structure (pl. 3, fig. 4) both in the light and dark grown cultures.

DISCUSSION

Based on the nature of chromatophores, the shape of the vegetative cells, and the manner of cell division, it is interesting to note that all the algal symbionts, isolated from the four species of lichens, belong to *Trebouxia* Group II.

The results show that the two different species of lichens contain the morphologically identical phycobionts (e.g. *A. comosa* and *A. dendricata*) and these differ physiologically only in their rate of growth under culture in the dark. The phycobiont of *A. comosa* grew faster than that from *A. dendricata* in dark culture on

agar-peptone dextrose used in the investigations. It seems that the lichen fungi are independent with regard to their algal symbionts.

Algal symbionts isolated from *P. caperata* and *P. rudecta* were similar to Ahmadjian's original isolates (1959b). Whereas, the algae isolated from *P. caperata* which were used in this study were different from those in my previous study (Wang-Yang, 1965). These differences are their physiological characteristics in culture. These show that the same species of lichen contain physiologically different forms of algal symbionts (e.g. *P. caperata* as compared in this study with my previous study, Wang-Yang, 1965). However, at the species level, lichen fungi are not highly specific with regard to their algal partners. The theory of the specificity of lichen with regard to their algal symbionts should be further investigated on the algal symbionts of lichens.

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